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The effect on immunity of long-term intensive training in elite swimmers

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SUMMARY

The impact of long-term training on systemic and mucosal immunity was assessed prospectively in a cohort of elite swimmers over a 7-month training season in preparation for national championships. The results indicated significant suppression ($P < 0.05$) of serum IgA, IgG and IgM and salivary IgA concentration in athletes associated with long-term training at an intensive level. There was also a trend towards lower IgG2 subclass levels in serum in athletes compared with controls ($P = 0.07$). There were no significant changes in numbers or percentages of B or T cell subsets, but there was a significant fall in natural killer (NK) cell numbers and percentages in athletes over the training season ($P < 0.05$). After individual training sessions there was a significant decrease in salivary IgA levels for athletes compared with controls ($P = 0.002$). In athletes there was a downward trend in salivary IgA levels over the 7-month training period in both the pre-exercise ($P = 0.06$) and post-exercise samples ($P = 0.04$). There were no significant trends in salivary IgG levels over the study period in either athletes or controls. The only significant change in salivary IgM levels was an increase in detection rate in the pre-competition phase in athletes ($P = 0.03$). The study suggests that training of elite athletes at an intensive level over both short- and long-time frames suppresses both systemic and mucosal immunity. Protracted immune suppression linked with prolonged training may determine susceptibility to infection, particularly at times of major competitions.

Keywords exercise immunity swimmers saliva immunoglobulins

INTRODUCTION

In recent years several reports have indicated that elite athletes are susceptible to upper respiratory tract infections (URTI), either immediately before or more importantly during major competitions [1–4]. It has been suggested that susceptibility to URTI is due to impaired immune function [3–6], but a direct link has not been established [7]. Exercise has been shown to alter various immune parameters at the time of exercise, giving rise to either enhanced or suppressed immunity depending on the intensity of exercise and fitness level of the study populations [5,6,8–10].

Studies of systemic immunity in sedentary and moderately exercising subjects have indicated enhanced immune responses following moderate exercise [8,9]. In contrast, highly trained athletes and sedentary individuals subjected to intense exercise have shown alterations in systemic immune parameters indicative of suppressed immunity during and immediately after training sessions [3,10–19]. While some changes are transitory and related to the acute effect of intense exercise, the changes in leucocyte numbers [14] and cytokine levels [18] in peripheral blood have been shown to persist for several days. Studies of mucosal immunity have also shown a decrease in salivary IgA levels in highly trained athletes following endurance exercise [3,20–25], but these studies have not been linked with an increased incidence of URTI.

Most studies have assessed the impact of exercise on immune parameters following individual episodes of intense exercise. This prospective study assessed the impact of long-term training at an intense level on systemic and mucosal immunity in a cohort of elite swimmers undertaking a 7-month training programme in preparation for national championships.

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SUBJECTS AND METHODS

Subjects

The study was conducted with the informed consent of the Australian Institute of Sport (AIS) Swimming Team, which

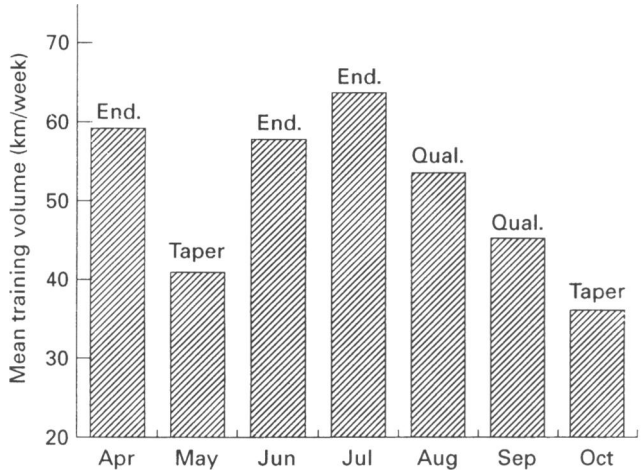

Fig. 1. Mean training volume per week (km/week) and monthly phase of training indicated as endurance (End), quality (Qual) and taper for the athletes over the training season.

consisted of 26 elite swimmers (15 males, 11 females) aged 16–24 years. The athletes were undertaking 20–25 h of pool training and 5 h of dry-land training per week in preparation for the World Championship Trials. The phase of training and average distances swum each week are shown in Fig. 1. Twelve AIS staff (seven males, five females) aged 19–41 years, who were involved in regular activity but limited to a maximum of 4 h per week of moderate exercise, acted as controls.

Study design

The athletes and controls were studied monthly from April to October, during the southern hemisphere autumn to spring. Blood for lymphocyte subsets and IgG subclasses was collected at the beginning of the study period and the lymphocyte subsets were repeated at the end of the study. Before a scheduled training session each month blood (from which serum was prepared) and saliva were collected from athletes and controls for immunoglobulin and albumin quantifications. Saliva was collected again immediately after the training session.

This study was designed to eliminate many of the physiological variables known to cause alterations in salivary immunoglobulin levels [26]. Saliva samples were collected at the same time each month, at least 1 h post-prandially, without stimulation, and a minimum of 24 h after the previous training session to reduce the physiological variability of salivary immunoglobulin levels due to flow rate, dehydration and prior exercise effects. Although temperature has been reported to have no effect on salivary IgA levels following exercise [27], any possible interaction was reduced by conducting all study sessions in the AIS indoor heated pool.

Blood tests

Blood was collected by standard venepuncture technique. Total leucocyte count and percentage of lymphocytes were measured on a Model Stks Coulter Counter (Coulter Electronics, Hialeah, FL). Commercial MoAbs (Coulter) were used to determine numbers and percentages of T cells and T subsets (CD3, CD4, CD8), B cells (CD19), natural killer (NK) cells (CD56) and an activation marker (HLA-DR) by flow cytometry using an EPICS II Flow Cytometer (Coulter). Serum immunoglobulins IgA, IgG, IgM and albumin were measured by rate nephelometry using a Beckman Array (Beckman, Brea, CA) analyser and Beckman antisera, controls and calibration material. The Beckman calibrators were referenced against WHO 67/95. Conversion factors to CRM470 are IgA × 0·99, IgG × 0·96, IgM × 0·95 and albumin × 1·04. IgG subclasses were measured by radial immunodiffusion using commercially prepared plates and reagents (The Binding Site, Birmingham, UK).

Salivary immunoglobulins and albumin

IgA, IgG, IgM and albumin were measured in unstimulated whole mixed saliva by electroimmunodiffusion [28] using commercially prepared IgA-specific antisera (Tago, Burlingame, CA) and IgG, IgM and albumin-specific antisera (Dako Immunoglobulins, Glostrup, Denmark). The assays were calibrated with Standard Human Serum referenced against WHO 67/95 (Behringwerke, Marburg, Germany). Conversion factors to CRM470 are IgA × 0·83, IgG × 0·85, IgM × 0·67 and albumin × 1·00.

Statistical analysis

Wilcoxon's risk sum test for unpaired data was used for comparisons between athletes and controls and between pre- and post-exercise levels on log-transformed data. Repeated monthly measurements for individuals were assumed to be independent. Changes over the training period were assessed for trends and by the difference between measurements taken in the first month and the last month for each athlete or control subject. Non-parametric statistical methods were used for all comparisons. $P < 0{\cdot}05$ was considered statistically significant.

RESULTS

Effects on systemic immunity

Lymphocyte subsets. The mean and s.d. of leucocyte counts and lymphocyte subsets collected at the beginning and end of the training season and comparison between levels in athletes and control subjects are shown in Table 1. There were no significant changes in total leucocyte counts, B cell and T cell subsets or HLA-DR activation markers in athletes or controls over the study period. The only significant difference at the commencement of the training season between athletes and controls was a marginally lower total leucocyte count in athletes compared with controls ($P = 0{\cdot}02$). There were no significant differences between athletes and controls at the end of the training season. The only significant change between subsets at the end of the training season compared with the beginning of training was a fall in NK cell numbers and percentages in athletes but not in control subjects. There was a significant decrease in NK cell percentage of total lymphocytes (mean change = 2·1% of cells) in athletes which represented a mean 35% decrease at the end of the study period ($P < 0{\cdot}05$). The decrease in NK cell total numbers in athletes (mean change = $0{\cdot}068 \times 10^9$ cells) represented a mean decrease of 57% in NK cell numbers ($P < 0{\cdot}05$).

Serum immunoglobulins and albumin. The monthly mean levels of serum IgA, IgG, IgM and albumin for athletes and controls are shown in Fig. 2 and the mean levels for all serum samples collected over the training season are given in Table 2.

Table 1. Comparison of the differences between athletes ($n = 25$) and controls ($n = 12$) for mean levels (and s.d.) of leucocyte count and lymphocyte subsets collected at the beginning and the end of the 7-month training season

		Commencement of training season			End of training season			Differences between start and end of training season		
Leucocyte subsets	Units	Athletes ($n = 25$)	Controls ($n = 12$)	P	Athletes ($n = 25$)	Controls ($n = 11$)	P	Athletes ($n = 25$)	Control ($n = 11$)	P
Total leucocyte count	$\times 10^9$ cells	6·47 (1·94)	7·33 (1·28)	0·02*	6·10 (1·64)	6·78 (0·88)	0·07	−0·36 (2·00)	−0·54 (1·22)	0·37
Lymphocyte no.	$\times 10^9$ cells	2·28 (0·57)	2·47 (0·53)	0·38	2·19 (0·50)	2·19 (0·43)	0·78	−0·13 (0·57)	−0·27 (0·50)	0·60
Lymphocyte (%)	% of leucocyte count	36·48 (7·90)	34·50 (8·57)	0·39	36·52 (8·36)	32·67 (6·29)	0·15	+0·04 (10·14)	−1·83 (7·48)	0·49
CD19 (B cells)	$\times 10^9$ cells	0·35 (0·16)	0·34 (0·19)	0·86	0·32 (0·14)	0·30 (0·15)	0·51	−0·03 (0·16)	−0·05 (0·11)	0·46
CD19 (%)	% of lymphocytes	15·32 (5·21)	13·83 (8·74)	0·23	14·96 (5·50)	13·73 (8·00)	0·15	0·36 (4·07)	−0·82 (2·64)	0·24
CD3 (T cells)	$\times 10^9$ cells	1·60 (0·39)	1·78 (0·48)	0·38	1·52 (0·42)	1·58 (0·45)	0·86	−0·08 (0·40)	−0·20 (0·34)	0·42
CD3 (%)	% of lymphocytes	70·60 (7·26)	71·33 (8·48)	0·70	70·44 (6·40)	70·83 (7·22)	0·96	−0·16 (6·13)	−0·50 (3·42)	0·61
CD4 (T helper)	$\times 10^9$ cells	0·94 (0·25)	1·02 (0·33)	0·65	0·93 (0·26)	0·99 (0·31)	0·80	−0·01 (0·22)	−0·06 (0·26)	0·66
CD4 (%)	% of lymphocytes	41·32 (7·31)	41·58 (9·31)	0·87	42·72 (6·26)	44·18 (6·63)	0·70	+1·40 (4·20)	+0·73 (6·39)	0·97
CD8 (T suppressor)	$\times 10^9$ cells	0·58 (0·19)	0·73 (0·33)	0·22	0·60 (0·16)	0·60 (0·15)	0·85	+0·01 (0·25)	−0·04 (0·08)	0·38
CD8 (%)	% of lymphocytes	27·32 (3·78)	28·92 (7·53)	0·59	28·88 (6·55)	27·27 (4·54)	0·97	+1·56 (6·34)	+0·09 (3·48)	0·12
CD4: CD8	Ratio	1·56 (0·41)	1·56 (0·60)	0·84	1·56 (0·36)	1·69 (0·48)	0·69	0·0 (0·29)	+0·03 (0·49)	0·38
CD56 (NK cells)	$\times 10^9$ cells	0·14 (0·11)	0·09 (0·04)	0·17	0·08 (0·05)	0·12 (0·07)	0·16	−0·07 (0·12)†	+0·03 (0·05)	0·06
CD56 (%)	% of lymphocytes	5·92 (4·28)	3·67 (1·50)	0·18	4·05 (2·92)	5·17 (2·40)	0·29	−2·10 (3·88)†	+1·17 (2·04)	0·03*
HLA-DR$^+$	$\times 10^9$ cells	0·39 (0·17)	0·38 (0·15)	0·88	0·38 (0·15)	0·35 (0·13)	0·45	−0·03 (0·11)	−0·03 (0·09)	0·82
HLA-DR$^+$ (%)	% of lymphocytes	17·04 (5·91)	16·08 (8·13)	0·42	17·52 (5·74)	16·58 (7·56)	0·31	−0·04 (2·65)	+0·50 (2·39)	0·83

* Significant difference between athletes and controls.

† Significant decrease in NK cell numbers and percentages at end of training season compared with commencement ($P < 0·05$).

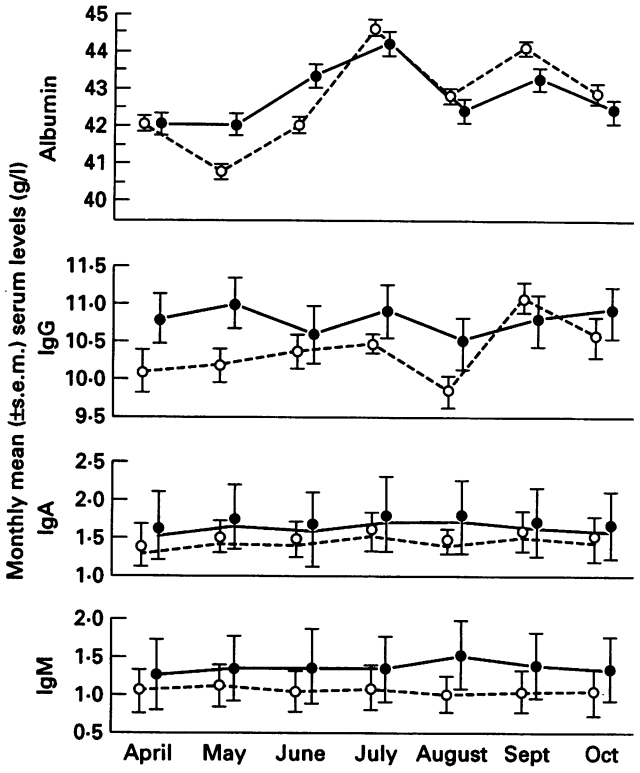

Fig. 2. Serum IgA, IgG, IgM and albumin levels (mean ± s.e.m.) for samples collected monthly from athletes (O) and controls (●) over the training season.

There were no significant differences between serum albumin levels in athletes compared with controls. Mean levels of serum IgA, IgG and IgM were all significantly lower in athletes compared with controls, and were not affected by age differences. There were no significant changes in serum IgG, IgM or albumin levels over the study period in either athletes or controls. Serum IgA levels did not alter significantly over the study period in controls, but the levels were slightly higher at the end of the training season in athletes. There was a 4·4% mean increase in serum IgA level in athletes, representing an average increase of 0·07 g/*l* (s.d. = 0·12, $P = 0·01$) from the start of the training season.

There were no significant differences between athletes and controls for any IgG subclass (Table 3), but there was a trend towards lower levels of IgG2 in athletes compared with controls ($P = 0·07$).

Effects on mucosal immunity

Salivary albumin. The pre-exercise and post-exercise salivary albumin levels remained stable over the study period in both athletes and controls. There were no significant differences for mean salivary albumin levels between athletes and controls for either pre- or post-exercise collections (Table 2). The salivary albumin levels on average decreased after individual training sessions in athletes, while in controls the salivary albumin levels increased, resulting in a significant difference ($P = 0·004$) for the changes from pre- to post-exercise levels between athletes and controls (Table 2).

Salivary IgA. There was a downward trend over the 7-month study period in both pre- and post-exercise salivary IgA

Table 2. Comparison of the mean (and s.d.) of serum and salivary immunoglobulins and albumin levels for all samples collected over the training season from other athletes and control subjects

Immune parameter	Units	Athletes ($n = 220$)	Controls ($n = 83$)	*P*
Serum IgA	g/*l*	1·51 (1·39)	1·73 (1·62)	0·01*
Serum IgG	g/*l*	10·28 (1·26)	10·80 (1·21)	0·03*
Serum IgM	g/*l*	1·07 (1·63)	1·38 (1·09)	0·0001*
Serum albumin	g/*l*	42·52 (1·08)	42·95 (1·09)	0·34
Pre-exercise:				
Salivary IgA	mg/*l*	50·40 (1·60)	41·70 (1·8)	0·05*
Salivary IgG	mg/*l*	8·10 (13·2)	3·90 (20·9)	0·05*
Salivary IgM	mg/*l*	0·03 (15·6)	0·01 (4·1)	0·01*
Salivary albumin	mg/*l*	35·20 (2·1)	30·90 (2·1)	0·23
IgA/albumin	ratio	0·70 (2·16)	1·35 (2·32)	0·70
Post-exercise:				
Salivary IgA	mg/*l*	45·20 (1·7)	46·10 (1·8)	0·72
Salivary IgG	mg/*l*	5·20 (18·5)	5·80 (14·7)	0·71
Salivary IgM	mg/*l*	0·02 (7·7)	0·01 (4·7)	0·39
Salivary albumin	mg/*l*	32·10 (2·2)	36·60 (2·0)	0·27
IgA/albumin	ratio	1·39 (2·23)	1·26 (2·18)	0·43
Post-pre-exercise changes:				
Salivary IgA	mg/*l*	0·90 (1·68)	1·09 (1·62)	0·002*
Salivary IgG	mg/*l*	0·64 (1·11)	1·48 (22·9)	0·07
Salivary IgM	mg/*l*	0·55 (16·8)	1·08 (3·86)	0·05*
Salivary albumin	mg/*l*	0·91 (1·99)	1·29 (1·82)	0·004*

* Significant difference between athletes and controls.

Table 3. Comparison of mean (and s.d.) for IgG subclass levels (g/l) in athletes and control subjects at the beginning of the study

IgG subclass	Athletes ($n = 26$)	Controls ($n = 12$)	P
IgG1 (g/l)	7·18 (2·52)	6·80 (1·48)	0·89
IgG2 (g/l)	2·57 (0·73)	3·54 (1·73)	0·07
IgG3 (g/l)	0·59 (0·21)	0·59 (0·15)	0·74
IgG4 (g/l)	0·53 (0·41)	0·36 (0·33)	0·17

levels in athletes (Fig. 3). The downward trend in salivary IgA over the study period for athletes approached significance for the pre-exercise levels ($P = 0·06$) and was significant for the post-exercise collections ($P = 0·04$). These trends were not observed in control subjects. The changes between the pre- and post-training session salivary IgA levels were consistent between months and showed no additional trends over the study period. The mean pre-exercise salivary IgA levels were significantly higher ($P = 0·05$) in athletes compared with controls, but the mean post-training session levels were not significantly different (Table 2). The salivary IgA level on average decreased after individual training sessions in athletes but increased in controls. The changes between pre- and post-exercise levels of salivary IgA were significantly different ($P = 0·002$) between athletes and controls, indicating significant decreases in IgA levels post-exercise for athletes.

There were no significant differences in the ratio of salivary IgA:salivary albumin between athletes and controls (Table 2). A downward trend over the study period was also observed for the salivary IgA:salivary albumin ratio in athletes, and approached significance for the pre-exercise ($P = 0·08$) and post-exercise saliva collections ($P = 0·06$).

Salivary IgG and IgM. There were no significant trends in the monthly mean salivary IgG or IgM levels over the study period in either athletes or controls. Pre-exercise levels of salivary IgG and IgM were significantly higher in athletes than in controls (Table 2). The post-exercise levels of salivary IgG and IgM were not significantly different between athletes and controls, but the changes between pre- and post-exercise levels indicated a significant decrease in IgM levels post-exercise in athletes ($P = 0·05$) and a similar trend for IgG ($P = 0·07$) (Table 2). The detection rate for salivary IgM was low throughout the study period, being 13% for samples collected from athletes and 15% from controls, though there was a significant increase ($P = 0·03$) in the detection rate (22%) in saliva collections from athletes in the pre-competition training phase. The IgM detection rate observed in saliva samples collected from controls during the same pre-competition period was 13%.

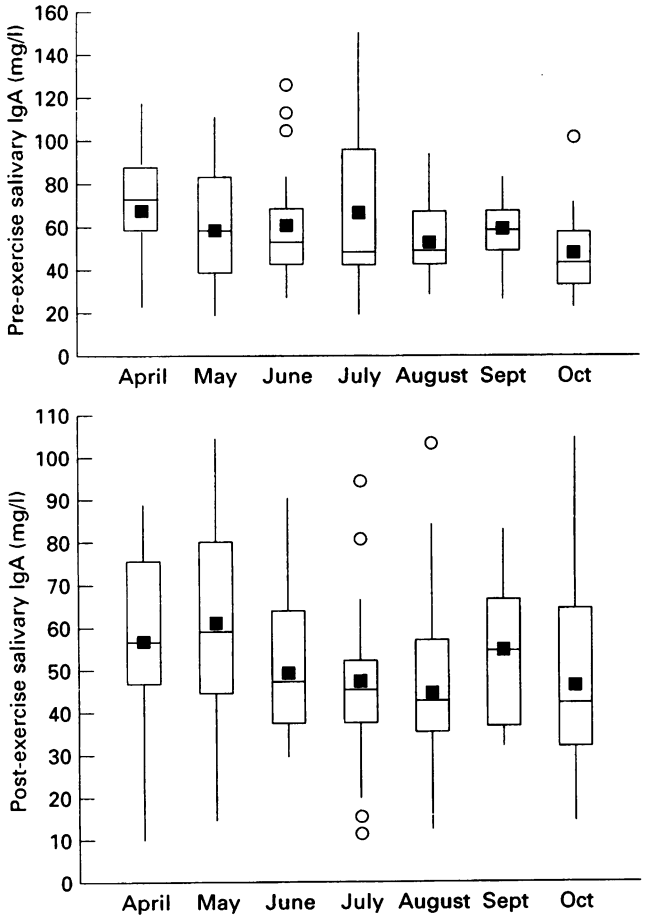

Fig. 3. Salivary IgA levels in pre-exercise and post-exercise samples collected at monthly intervals from athletes and for all samples collected over the training season. The whisker-box plots represent the 25th–75th percentile of results inside the box; the median is indicated by a bar across the box and the mean by a solid black square (■). The whiskers on each box represent the 5th–95th percentile of results and O represents outliers.

DISCUSSION

This study of highly trained elite swimmers showed suppression of immune parameters in athletes compared with control subjects associated with long-term training. The significant changes in athletes included: (i) lower serum IgA, IgG and IgM levels and a trend towards lower levels of IgG2 subclass; (ii) a decrease in mean salivary IgA levels after individual training sessions; (iii) a fall over the 7-month training season in NK cell numbers and percentage of lymphocytes and both pre-exercise and post-exercise salivary IgA levels. There were no differences in peripheral blood B cells, T cell subsets or HLA-DR$^+$ populations between athletes and controls, nor were there significant changes in these parameters over the 7-month training period.

Although serum immunoglobulin levels in the athletes were within normal population reference ranges, the levels of all three major classes were below the 10th percentile. The mean serum IgA, IgG and IgM levels in athletes were all significantly lower than for control subjects. One explanation for the lower levels of serum immunoglobulins in athletes compared with controls is that the years of training at an elite level may result in a small, in absolute terms, but significant chronic suppression of systemic immunity. Serum IgG levels decreased with increasing distances swum by the athletes (M. Gleeson *et al.*, unpublished observation), and the suppression of total IgG levels was reflected mainly in lower levels of IgG2 subclass. Low

levels of IgG2 have been previously reported in long distance runners [13], where the differences in IgG subclasses were also noted to be small on an absolute scale. Ricken & Kindermann [29] have also shown that low levels of serum immunoglobulins are associated with an increased susceptibility to infection. This long-term study was initated by our observation of hypogammaglobulinaemia in one elite swimmer which reversed with a reduced training schedule (P. Fricker *et al.*, unpublished observation), suggesting that some individual athletes may develop a protracted systemic immune deficiency.

The current study also showed a significant fall in NK cell numbers and NK percentage of lymphocytes in the athletes over the 7-month training season, suggesting a further chronic effect of long-term intensive training. A fall in NK cell numbers after exercise has been reported by other investigators studying the acute effects of exercise on immunity [12,14,19,21] and may reflect a physiological redistribution of NK cells out of peripheral blood during intensive physical training [17]. However, it has also been shown that levels of NK cell numbers return to normal within 24 h [12–17,19]. As the blood collections in this study were taken more than 24 h after the previous training session, the decrease in NK cell numbers and percentages are likely to reflect a protracted suppression rather than an acute effect. Whether the changes in NK cell numbers are an acute or chronic effect of exercise, the fall has the potential to leave an athlete susceptible to viral infection. NK cell activity was not addressed in this study, and further investigations are required to assess the significance of this decrease in NK cells in elite swimmers.

It has been previously reported that salivary IgA levels are decreased immediately following intense exercise [16,20–25] in other sporting events. This study confirms these observations of a fall in salivary IgA after individual sessions of intense exercise in elite swimmers, but in addition documents a significant fall in salivary IgA levels in these athletes over a long-term training period. The mean salivary albumin levels and ratios of IgA:albumin in saliva did not alter over the study period in athletes or controls, confirming that the observed decrease in salivary IgA levels was not due to alterations in flow rate or dehydration in the athletes. The results indicate that intense training chronically suppresses salivary IgA levels in addition to suppression of systemic immunity.

Detection of salivary IgM in athletes was most frequent in the pre-competition phase at the end of the training season, suggesting that IgM secretion may be a compensation mechanism for suppressed salivary IgA following long-term training. IgM is known to act as a compensation mechanism in mucosal secretions of IgA-deficient subjects [30], as a result of the increased proportion of IgD- and IgM-producing cells at mucosal surfaces in these subjects [31]. Failure of IgM compensation may increase the susceptibility of respiratory infection in elite athletes, and requires further investigation.

This study provides evidence of chronic suppression of both systemic and mucosal immunity in a cohort of elite athletes. The mechanism of this immunosuppression is unknown, but may be mediated by hormonal changes associated with exhausting exercise [32]. The observations provide a framework for assessment of those athletes who present with fatigue and an apparant infection-prone state. These athletes may have a reduction of serum and secretion immunoglobulins or levels at the lower end of the population reference range. Further studies are in place to determine the clinical significance of the current observations, the mechanisms of immune suppression, and implications for training and management strategies to prevent or reverse exercise-induced immune suppression.

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